

Supplementary Info

Supplementary Figure Legends:

Supplementary Figure 1. Mad2 levels in lungs of studied genotypes. a, quantitative western blot of lung tissue from CCSP-rtTA, TI-M, TI-K and TI-KM on doxycycline. b, HA immunohistochemistry of lung sections from TI-M and TI-KM mice on doxycycline to detect TetO-Mad2 transgene. c, HA immunohistochemistry of lung tumors from TI-K and TI-KM mice on doxycycline to detect TetO-Mad2 transgene in tumors. Scale bar; blue 100 μm , red: 1mm.

Supplementary Figure 2. Lung Tumors from TI-K and TI-KM Mice are Positive for Type II Pneumocyte Markers. Positive immunostaining of Prosurfactant protein C (SP-C) in lung tumor cells from both TI-K and TI-KM mice (a, b). Clara cell (CC26) staining of the tumors of TI-K and TI-KM mice showing negative staining in tumor cells (c,d) and positive cells in the airways (e, f). Black bar: 100 μm , red bar: 20 μm .

Supplementary Figure 3. Kras/Mad2-Induced Lung Tumors Show Increased Heterogeneity Compared to Kras-Induced Tumors. Consensus clustering of 14 TI-KM and 8 TI-K lung tumor expression array showing tighter clustering between primary tumors from TI-K mice and less tight clustering between tumors from TI-KM mice. For the consensus clustering algorithm, we randomly picked a different subset of the genes' weight by their coefficient of variation over the samples for 10,000 iterations. This subset of genes was then clustered using standard hierarchical clustering with the correlation

distance measure and complete linkage. At each iteration we keep track of what samples were in the sample cluster and compute the consensus matrix M_{ij} which is equal to the fraction of times sample i and j are in the same cluster. The heatmap on the right plots this matrix (from white=0 to bright blue=1). Finally, to pick out robust cluster groups we clustered the M_{ij} matrix again with the hierarchical algorithm this time with the standard Euclidean distance. The heatmap on the left is a plot of the actual expression data where each gene has been centered so its mean over the samples is zero.

Supplementary Figure 4. MR images and histological sections of lungs from TI-K (a) and TI-KM (b) mice on doxycycline (left). After two, three or four weeks of doxycycline withdrawal (middle) lung opacities disappeared from MR images, and the tumors regressed histologically (right). Red asterisk shows location of the tumors. Arrows show fibrosis, presumably where tumors were located. H: heart. Scale bar: black 200 μm , blue 100 μm and red 1mm. The total tumor volume is indicated in yellow.

Supplementary Figure 5. Histological sections of lung from TI-K and TI-KM mice on doxycycline for 5 weeks showing that no tumors are present (top). After 5 weeks on doxycycline, mice were placed on normal diet for 35 additional weeks and analyzed for the presence of tumors (middle and bottom). Scale bar: black 200 μm , blue 100 μm .

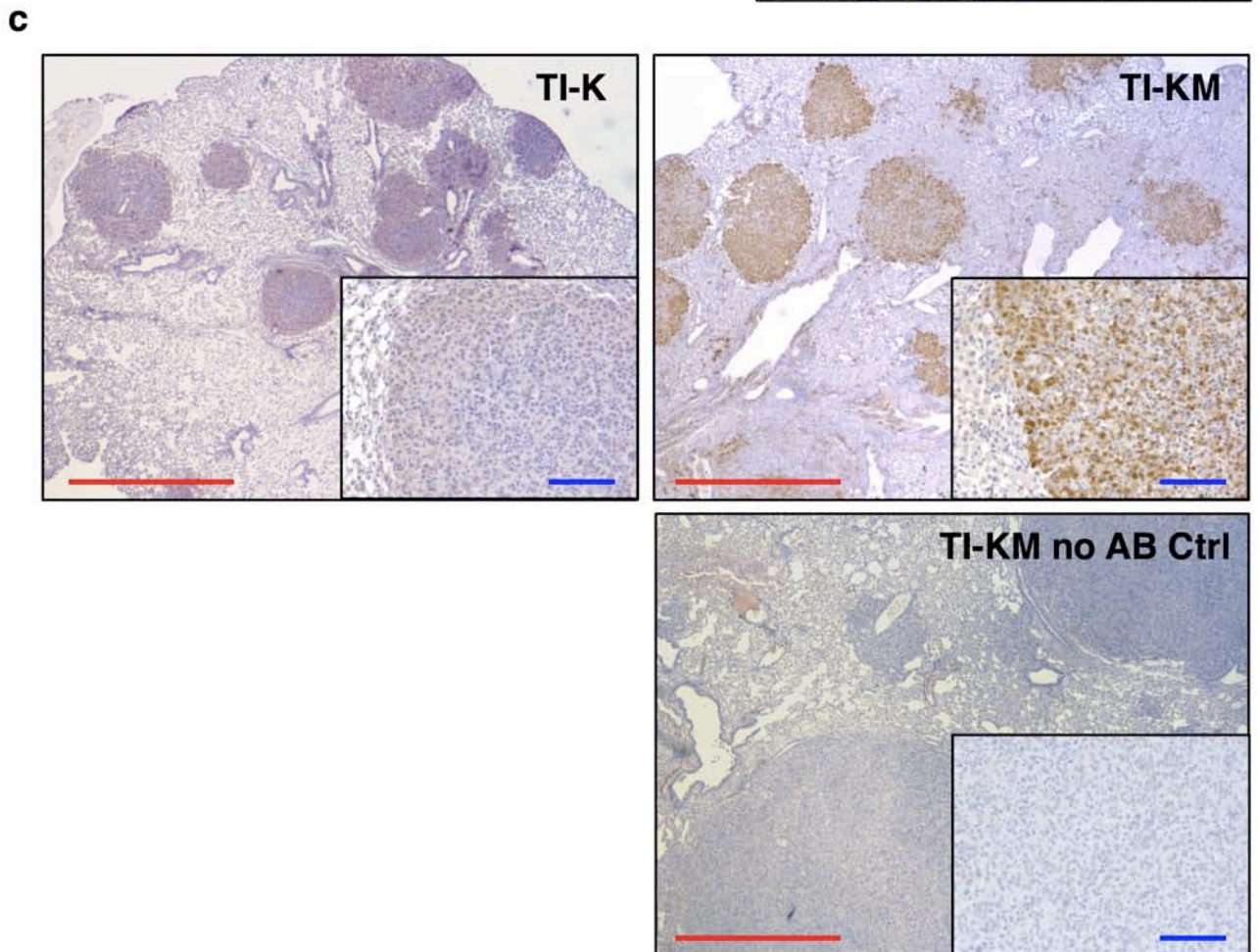
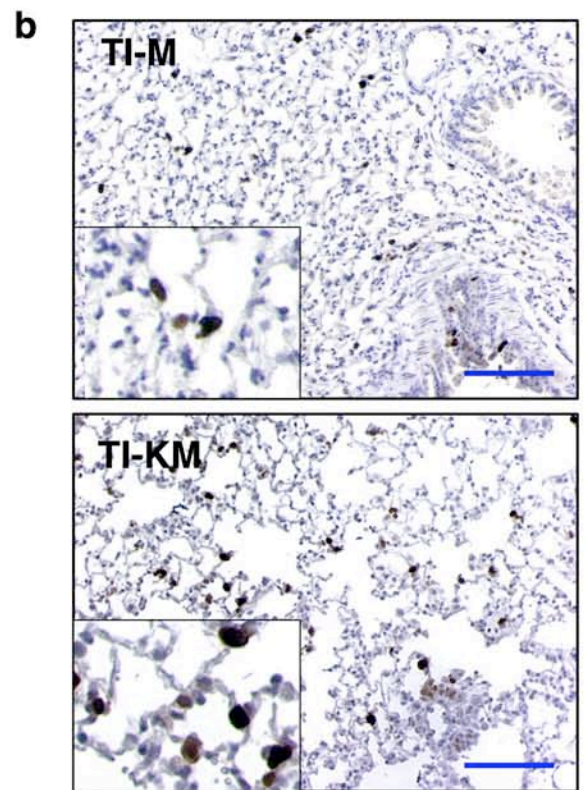
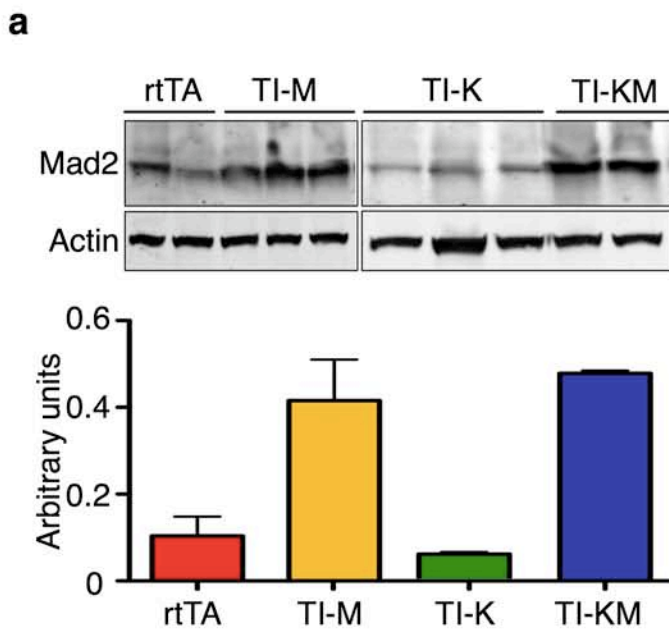
Supplementary Figure 6. a, Hematoxylin and Eosin stained sections of lung samples derived from TI-K or TI-KM mice on doxycycline showing the nuclear morphology of the tumor cells. b, HA specific immunohistochemistry of different recurrent tumors

showing positive staining in only 1 of 6 recurrences. Scale bar: red 1mm, black 200 μm , blue 100 μm , yellow 30 μm .

Supplementary Figure 7. Primary Tumors from TI-K and TI-KM Mice are positive for the MAPK, Stat3 and Pi3K Pathway. Immunohistochemistry of pERK, pStat3, pAKT and pS6 in tumor samples from TI-K and TI-KM mice on doxycycline showing positive staining for all 3 pathways in all the tumors analyzed. Scale bar: black 500 μm , blue 100 μm .

Supplementary Figure 8. Immunohistochemistry of pERK, pStat3 and pAKT in tumor relapses showing positive staining for pERK in relapse 2, 10 and 11, low levels of pstat3 in relapses 2, 10 and 11 and positive staining for pAKT in all of them. Scale bar: 100 μm .

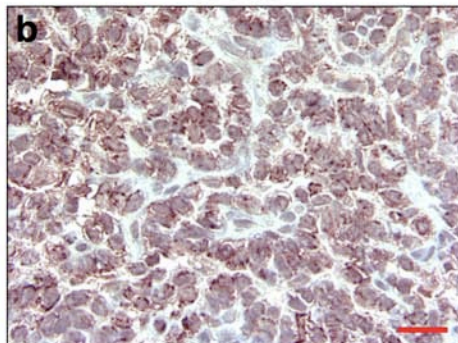
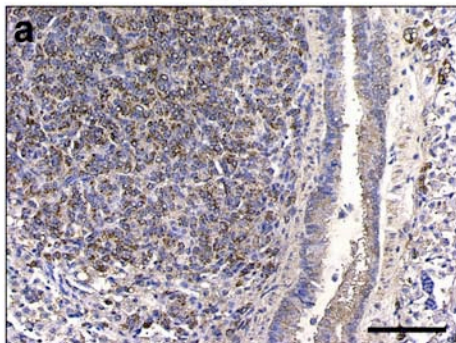
Supplementary Figure 9. a, Dendrogram and Heatmap of expression arrays from primary TI-K and TI-KM tumors as well as several TI-KM relapses (R) showing that relapses are very heterogeneous and do not cluster in defined groups. b, Consensus clustering of TI-K and TI-KM primary tumors and TI-KM relapse tumors. For details of methods see Supplementary Figure 3 legend.



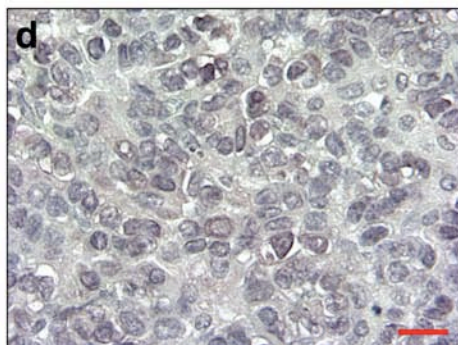
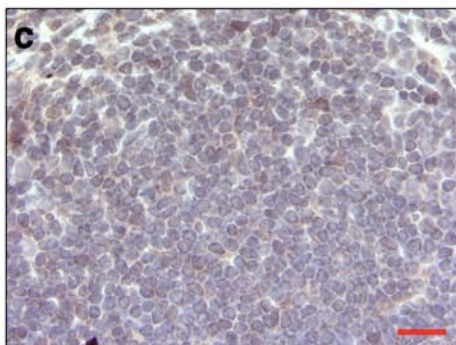
TI-K

TI-KM

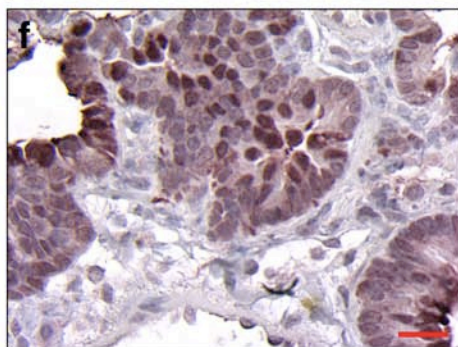
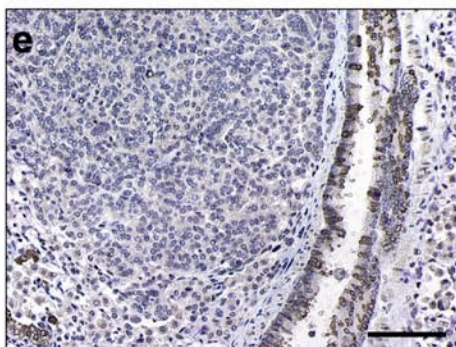
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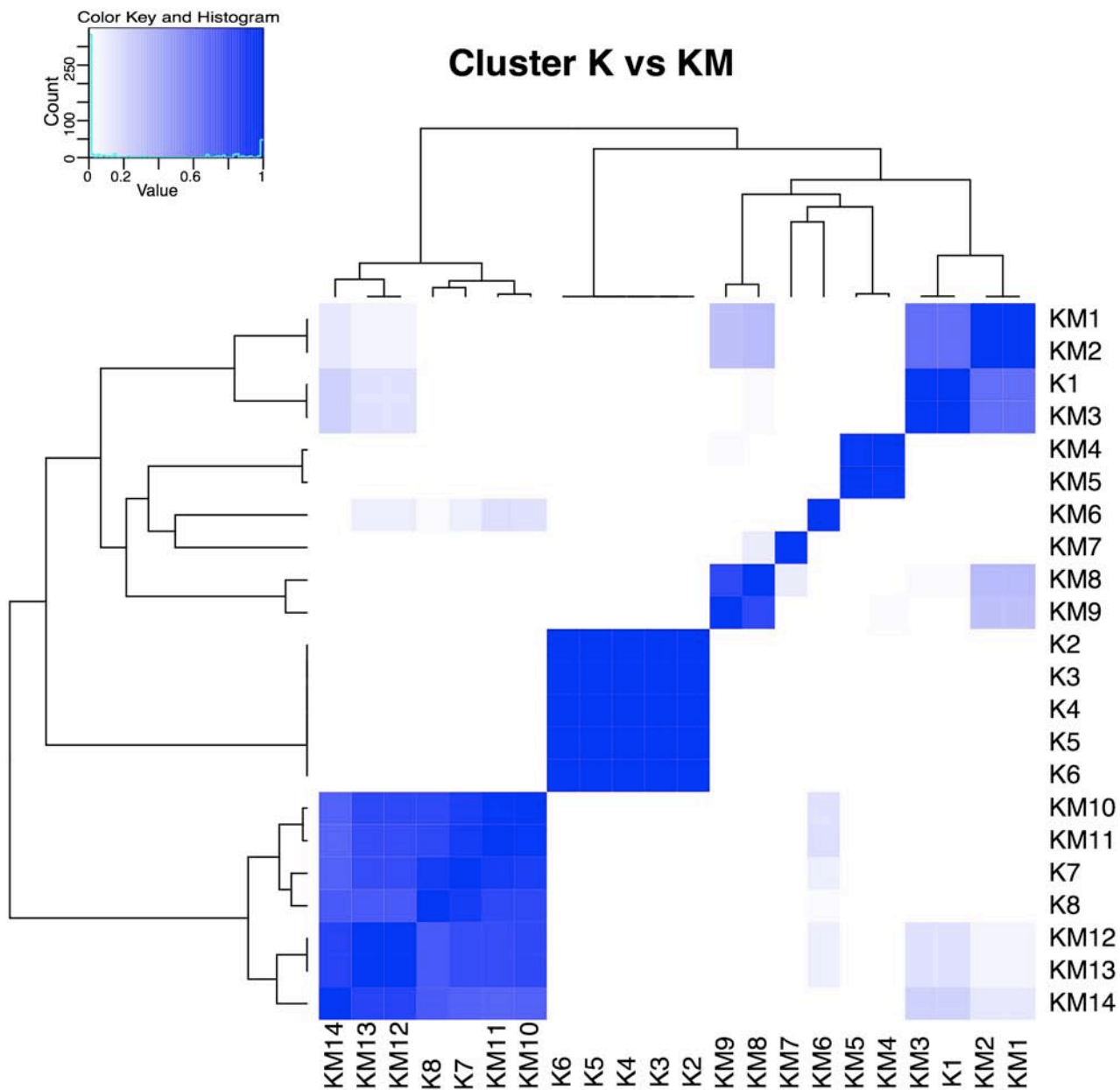


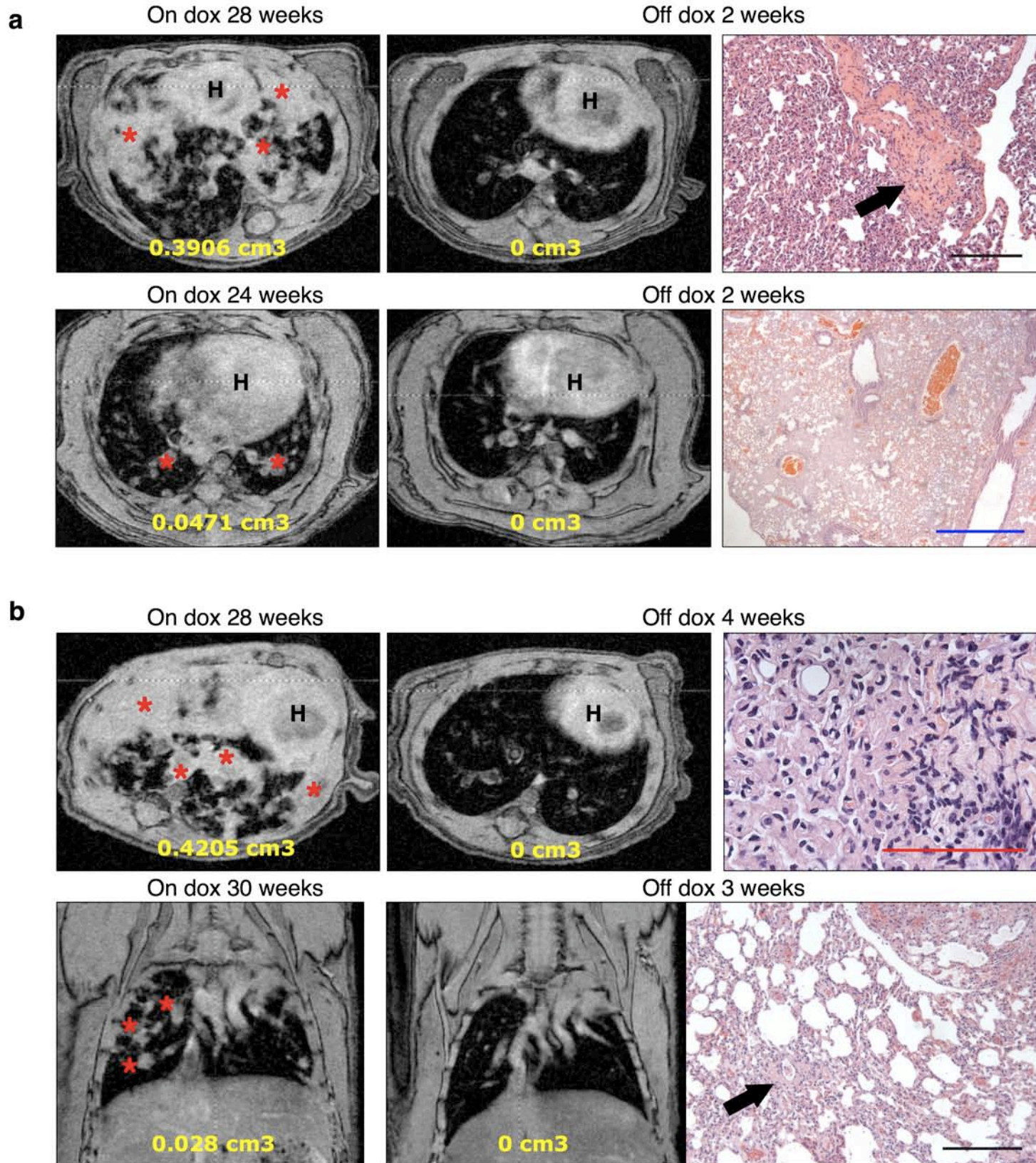
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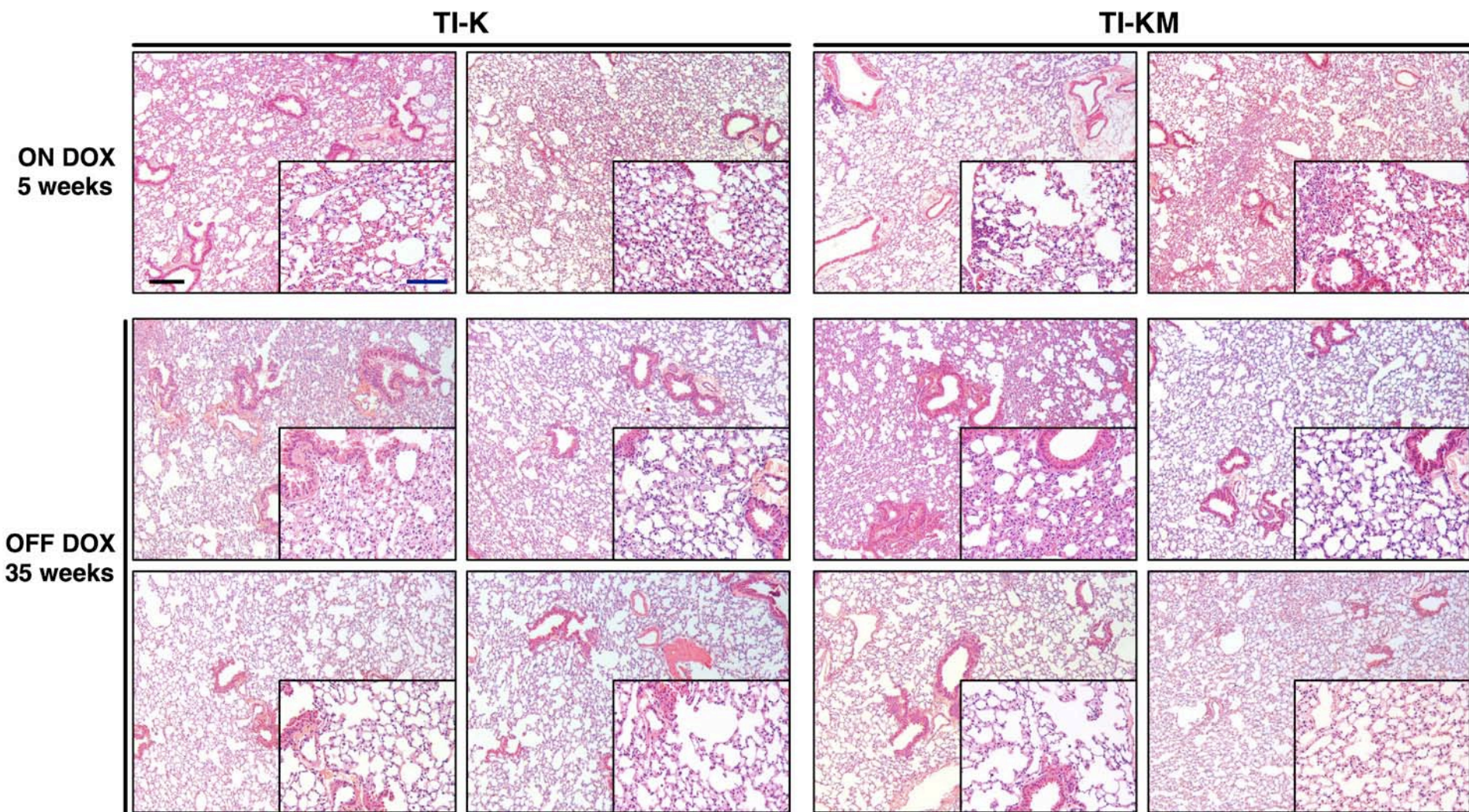


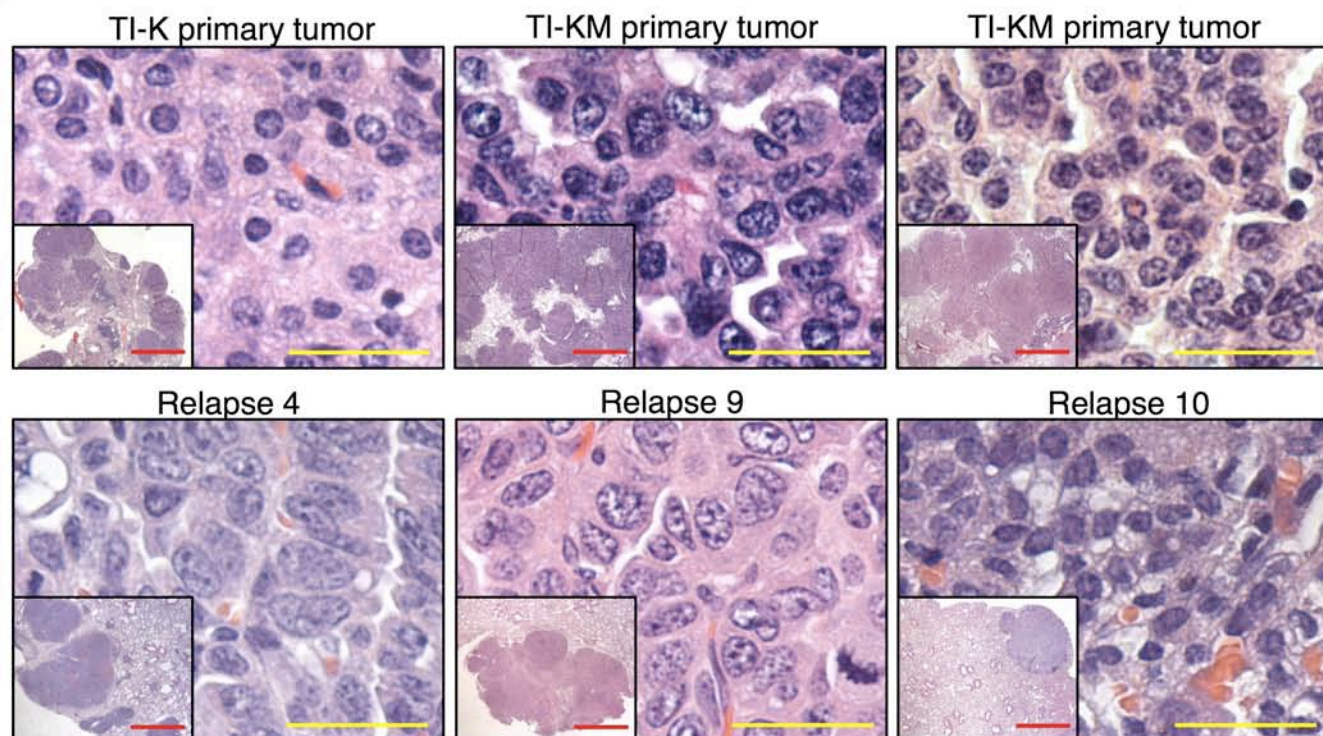
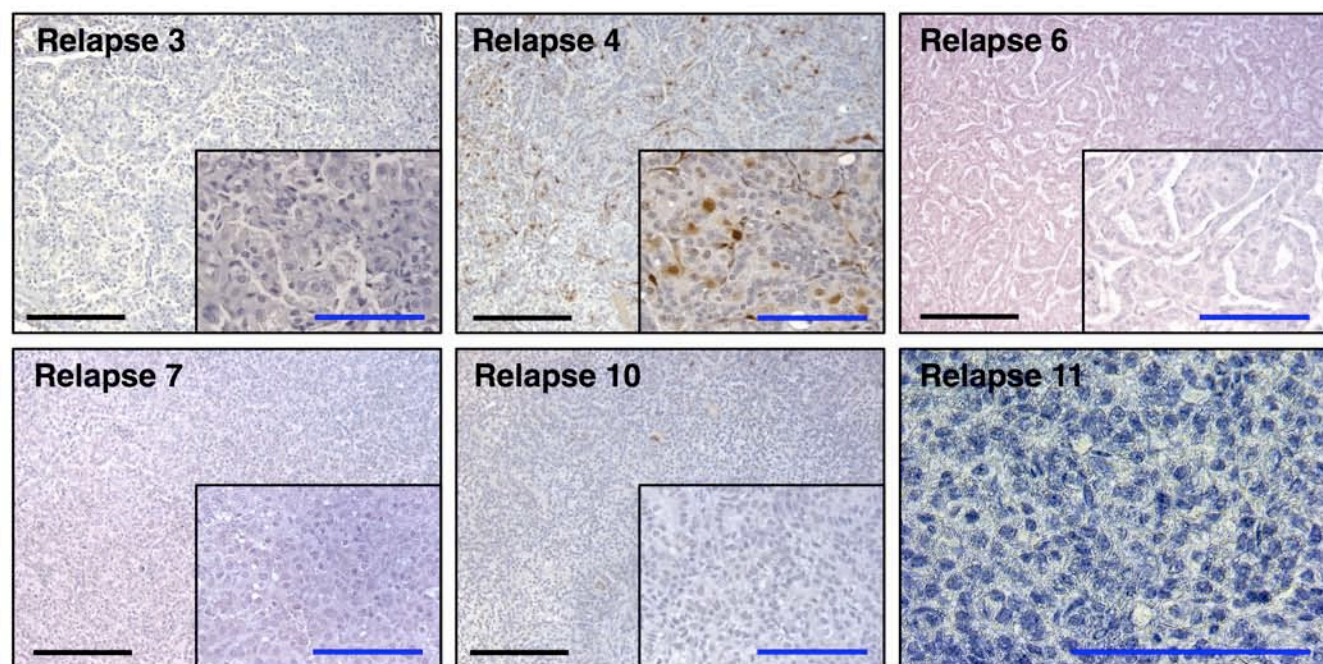
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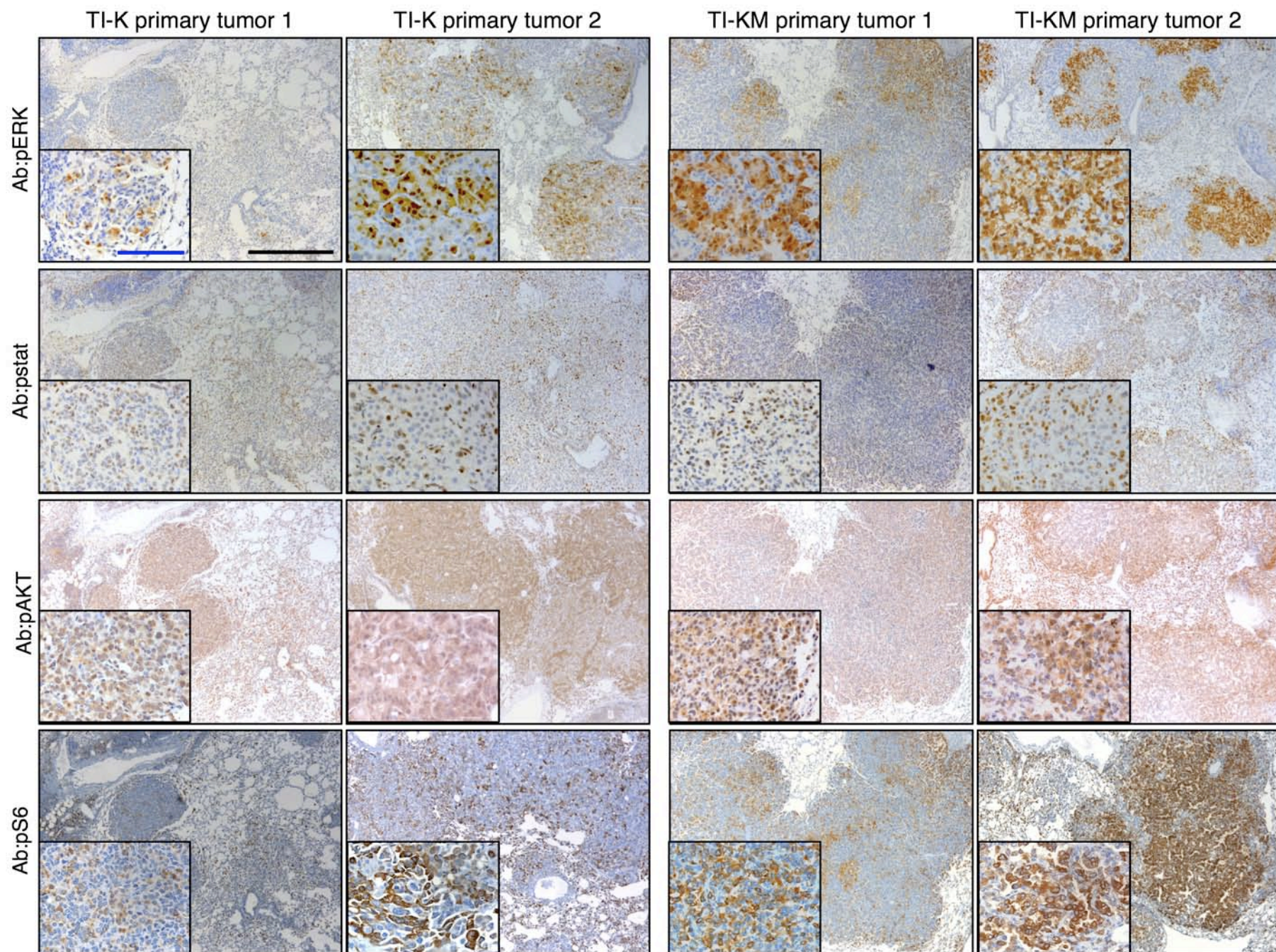




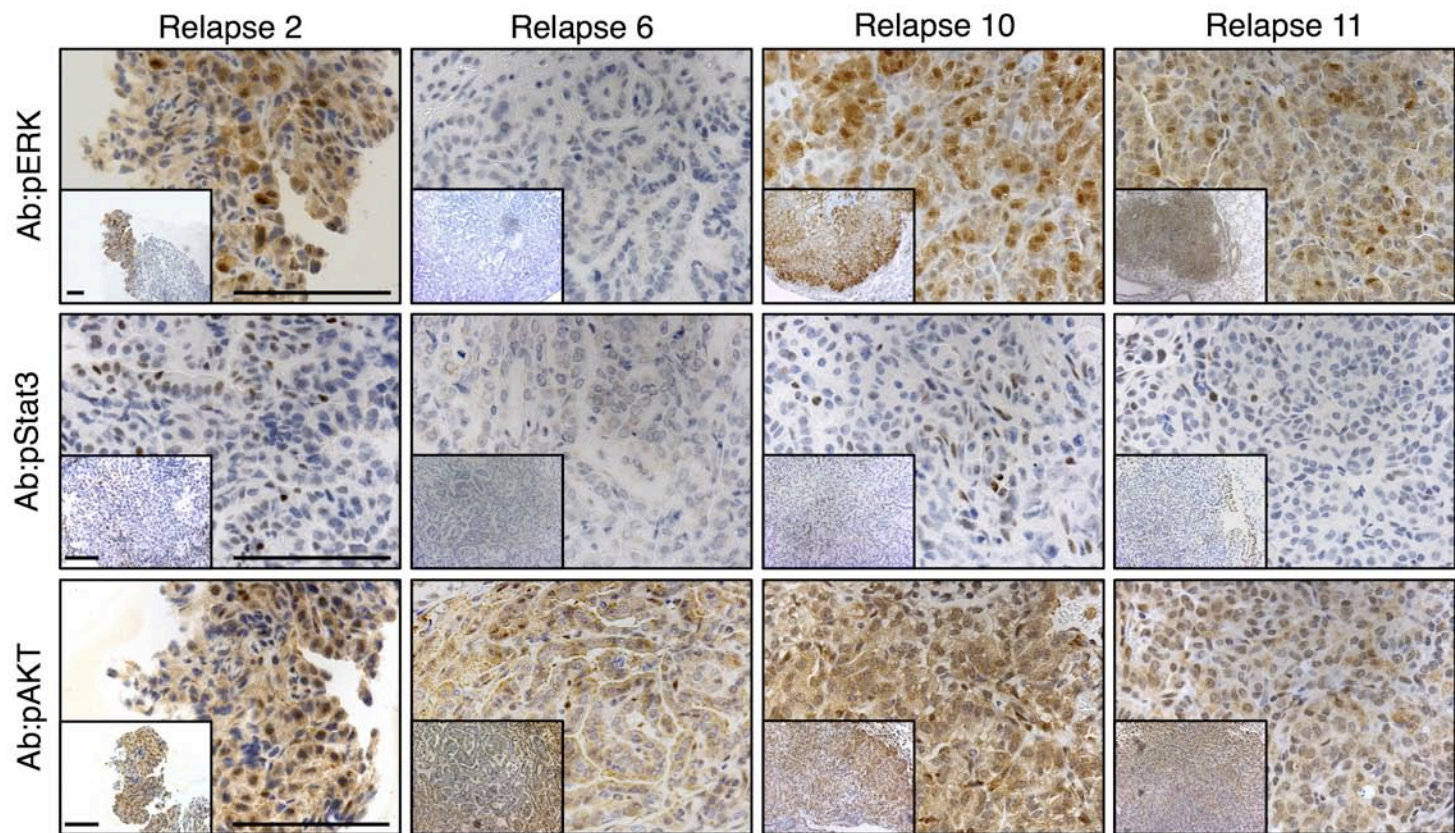




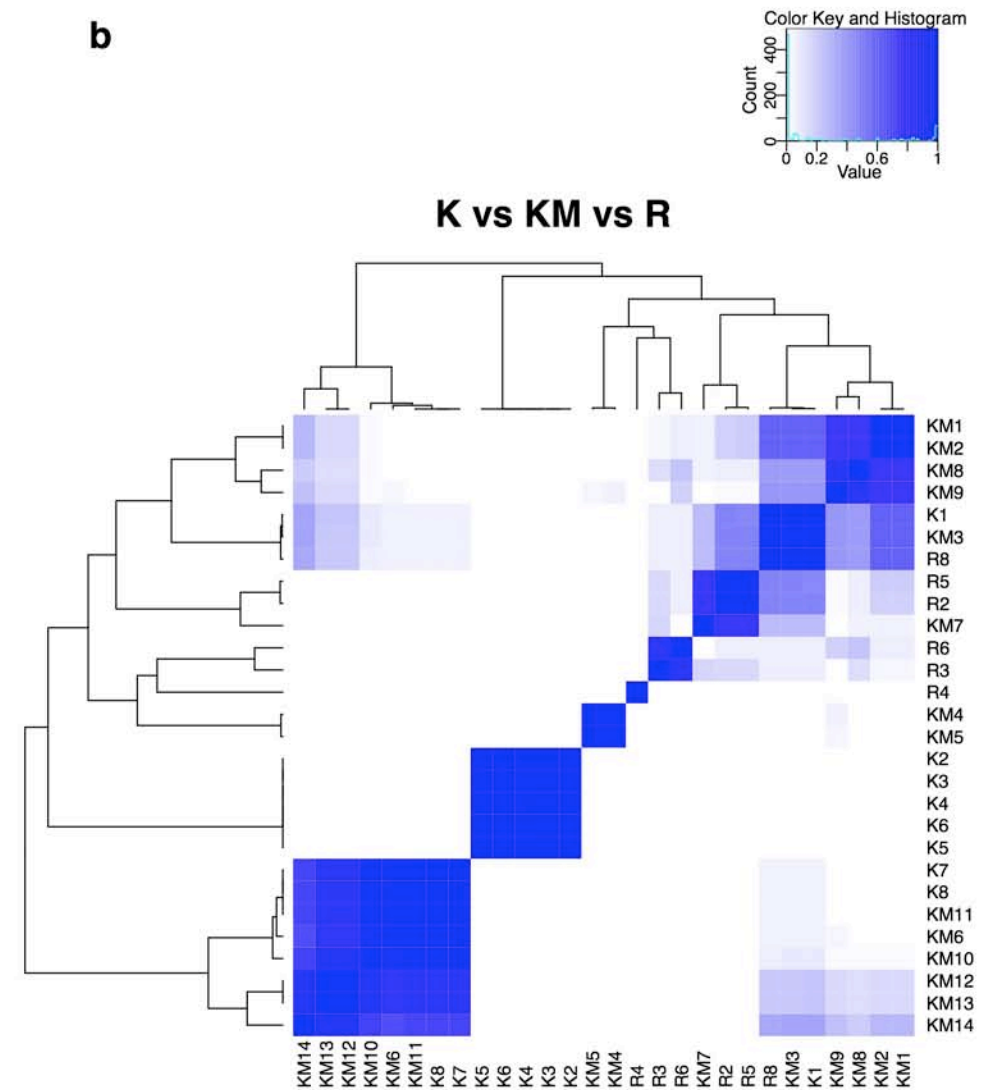
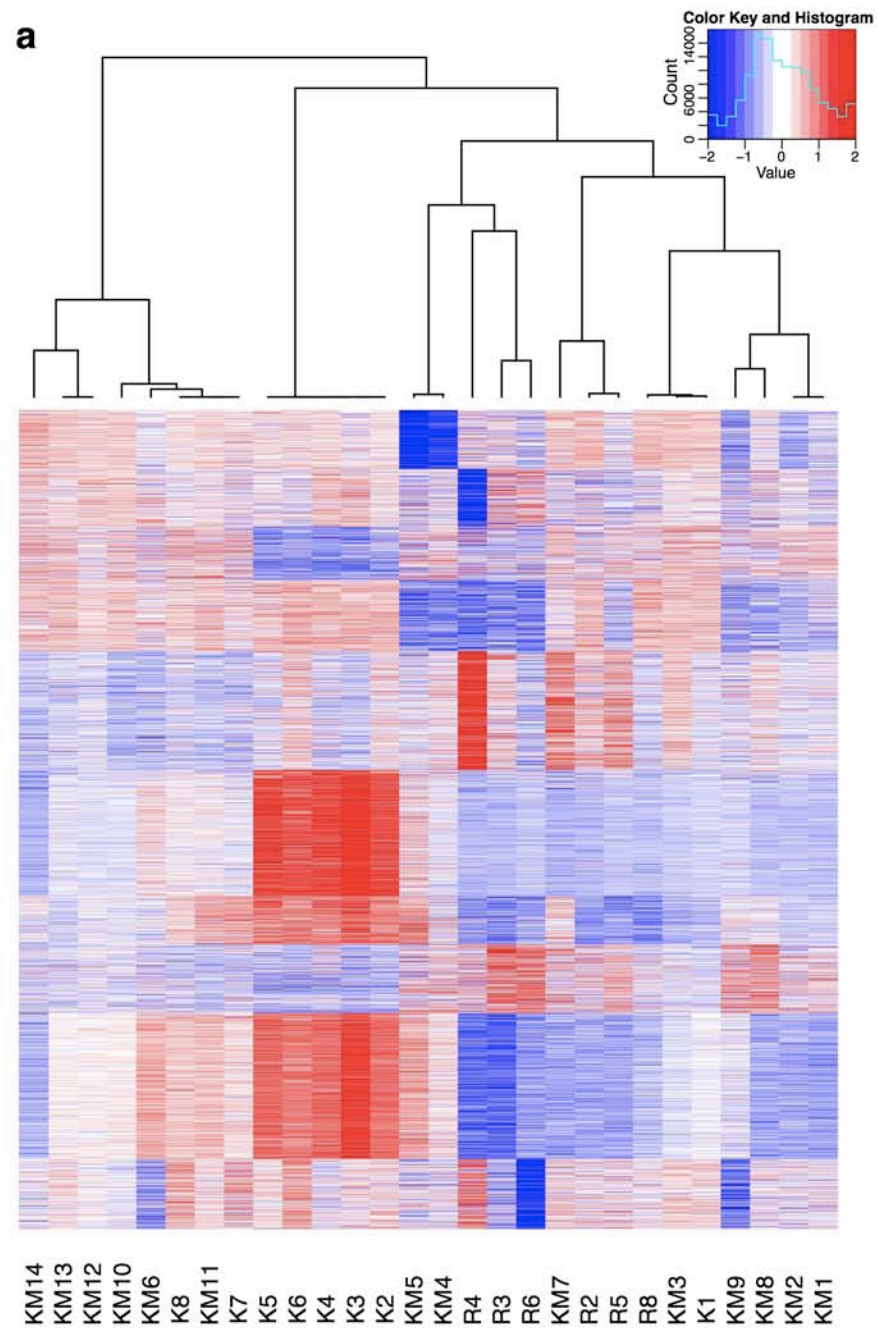
a**b**



Sotillo_Supplementary fig. 7



Sotillo_Supplementary fig. 8



Supplementary Table 1. Tumor Onset and Regression in TI-K and TI-KM Mice.

Mouse #	Genotype	Weeks On Dox (MRI status)	Weeks Off Dox (MRI status)
#1	TI-K	18 (Tumor)	2 (no tumor)
#2 ^a	TI-K	18 (Tumor)	2 (no tumor)
#3 ^b	TI-K	28 (Tumor)	2 (no tumor)
#4	TI-K	24 (Tumor)	2 (no tumor)
#5 ^c	TI-K	24 (Tumor)	2 (no tumor)
#6	TI-K	24 (Tumor)	2 (no tumor)
#7	TI-K	35 (Tumor)	4 (no tumor)
#8 ^a	TI-KM	18 (Tumor)	2 (no tumor)
#9	TI-KM	18 (Tumor)	2 (no tumor)
#10	TI-KM	27 (Tumor)	4 (no tumor)
#11	TI-KM	28 (Tumor)	2 (no tumor)
#12 ^d	TI-KM	28 (Tumor)	2 (no tumor)
#13	TI-KM	31 (Tumor)	4 (no tumor)
#14	TI-KM	31 (Tumor)	4 (no tumor)
#15 ^e	TI-KM	30 (Tumor)	3 (no tumor)
#16	TI-KM	30 (Tumor)	2 (no tumor)

^a MR Images from these mice are shown in Fig. 2c; ^{b,c} Supp. Fig 4a;

^{d,e} Supp. Fig 4b.

Supplementary Table 2. Phenotype in TI-K and TI-KM Mice On and After Doxycycline Withdrawal.

Genotype	ON DOX		OFF DOX					Histology
	wks	MRI I (wks)	MRI II (wks)	MRI III (wks)	MRI IV (wks)	MRI V (wks)	Sacrificed (wks)	
TI-K	12	T (12)	CR (10)				32	Pleural fibrosis
	12	T (12)	CR (14)	CR (36)			50	Pleural fibrosis
	12	T (12)	CR (14)	CR (36)			36	Normal lung tissue
	15		CR (22)				37	Pleural fibrosis
	15		CR (22)				37	Pleural fibrosis
	16			CR (20)	CR (35)		56	Pleural fibrosis
	17		CR (22)				26	Normal lung tissue
	17		CR (22)	CR (37)			37	Fibrosis
	18	T (18)	CR(2)				32	Fibrosis
	20		CR (5)	CR (26)			31	Normal lung tissue
	28	T (28)	CR (2)				20	Normal lung tissue/Scarring
	37		CR (2)	CR (12)			16	Fibrosis
	37		CR (2)	CR (12)			24	Normal lung tissue
	37		CR (2)	CR (12)			16	Fibrosis
	24	T (24)	CR (2)	CR (7)	CR (11)	CR(15)	16	Fibrosis
	22	T (22)	PR* (2)	CR (7)	CR (11)	CR (15)	15	Died during MRI
	24	T (24)	PR (2)	CR (8)	CR (12)	CR (16)	21	Fibrosis
	24	T (23)	CR (2)	CR (6)	CR (12)	CR (16)	22	Fibrosis ¹
	15	T (15)	CR (2)	CR (9)			36	Normal lung tissue
	25	T (25)	CR (2)	CR (7)	CR (11)	CR(19)	25	Fibrosis
	22	T (22)	PR (2)	CR (7)	CR (11)	CR(19)	25	Fibrosis
	23	T (23)	CR (3)	CR (9)	CR* (17)	CR (20)	28	Fibrosis
	26	T (26)	PR (2)				2	Died during MRI
	22	T (22)	CR (2)	CR (9)	CR (17)	CR (27)	37	Fibrosis
	26	T (26)	CR (3)	CR (8)	CR (16)	CR (22)	35	Fibrosis
TI-KM	11	T (11)	CR (14)	CR (36)			36	Normal lung tissue
	16		CR (20)	CR (36)			56	Fibrosis
	27		CR (2)	CR (12)			21	Normal lung tissue/Scarring
	20	T (20)	CR (5)	CR (27)			31	Normal lung tissue
	17		CR (22)				37	Pleural fibrosis
	19	T (19)	CR (2)	CR (32)			32	Parenchymal fibrosis
	28	T (28)	CR (2)	CR (20)			20	Fibrosis
	48	T (48)	CR (12)				21	Normal lung tissue/Scarring
	37		CR (12)				16	Parenchymal fibrosis
	22	T (22)	PR (2)	CR (6)	CR (9)		9	Normal lung tissue/Scarring
	26	T (26)	CR (2)	CR (8)	CR (17)		20	Pleural fibrosis
	25	T (25)	CR (3)	CR (9)			9	Died during MRI
	20	T (20)	CR (2)	CR (8)	CR (12)		12	Died during MRI
	23	T (23)	CR (4)	CR (8)	T (20)		20	Adenoma (R1)
	23	T (23)	CR (2)	CR (6)	T (16)	T (20)	22	Adenocarcinoma ² (R2)
	20	T (20)	CR (2)				44	Adenoma (R3)
	22						40	Adenoma (R4)
	31	T (31)	CR (12)				16	Adenocarcinoma (R5)
	35		CR (12)				16	Adenocarcinoma (R6)
	28	T (28)	CR (2)	T (10)			32	Adenocarcinoma (R7)
	28	T (28)	CR (2)	CR (6)	T (14)	T (18)	22	Adenocarcinoma ³ (R8)
	24	T (24)	PR (3)	CR* (9)	CR (17)	T (20)	23	Adenoma (R9)
	20		CR (5)	T (27)			31	Adenoma (R10)
	11	T (11)		T (14)	T (36)			Adenoma (R11)

CR: Complete regression. PR: Partial regression. T: Tumor. R: relapse. * artifactual image

¹Images from this mouse are shown in Fig. 3a upper panel, ² middle panel, ³ lower panel

Supplementary Table 3. Tumor volumes and times of induction and de-induction of primary tumors and corresponding relapses.

Mouse ID	Genotype	Weeks On Dox	Volume (cm3)	Weeks Off Dox	Volume (cm3)	Weeks Off Dox	Volume (cm3)	Weeks Off Dox	Volume (cm3)
#1	TI-K	12	0.052	14	0	36	0		
#2	TI-K	12	0.052	14	0	36	0		
#3	TI-K	18	0.400	2	0				
#4	TI-K	28	0.391	2	0				
#5	TI-K	22	0.047	2	0.0053	7	0		
#6	TI-K	24	0.323	2	0.0190	8	0		
#7 ^a	TI-K	23	0.047	2	0	6	0		
#8	TI-K	15	0.033	2	0	9	0		
#9	TI-K	24	0.151	2	0	7	0		
#10	TI-K	22	0.375	2	0.0062	7	0		
#11	TI-K	23	0.174	3	0	9	0		
#12	TI-K	26	0.102	2	ND				
#13	TI-K	22	0.134	2	0	9	0		
#14	TI-K	26	0.056	3	0	8	0		
#15	TI-K	24	0.098	2	0	7	0		
#16	TI-KM	11	0.034	14	0	36	0		
#17	TI-KM	19	0.289	2	0	32	0		
#18	TI-KM	20	0.322	5	0	27	0		
#19	TI-KM	28	0.425	2	0	20	0		
#20	TI-KM	25	0.275	3	0	9	ND		
#21	TI-KM	20	0.067	2	0	8	0		
#22	TI-KM	22	0.075	2	0.0220	6	0		
#28 (R1)	TI-KM	23	0.049	4	0	20	0.0113		
#29 ^b (R2)	TI-KM	23	0.032	2	0	16	0.0130	20	0.0133
#23 (R3)	TI-KM	20	0.022	2	0				
#27 (R5)	TI-KM	31	0.107	12	0				
#24(R7)	TI-KM	28	0.052	2	0	10	0.0154		
#30 ^c (R8)	TI-KM	28	0.147	2	0	14	0.0121		
#26 (R9)	TI-KM	24	0.291	3	0.0119	9	0	20	0.0199
#25 (R11)	TI-KM	11	0.042	14	0.0126	36	0.0228		

^a MR Images from this mouse are shown in fig 3a (upper panel), ^b (middle panel) and ^c(lower panel).